PB86-168515

Spot Test for Polycyclic Aromatic Hydrocarbons

(U.S.) Industrial Environmental Research Lab.
Research Triangle Park, NC

1982

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. PB86-168515
EPA/600/D-82/082		

4. TITLE AND SUBTITLE	5. REPORT DATE
A Spot Test for Polycyclic Aromatic Hydrocarbons	1982
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
L. D. Johnson, R. E. Luce, and R. G. Merrill	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
See block 12	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
EPA, Office of Research and Development Industrial Environmental Research Laboratory Research Triangle Park, NC 27711	Symposium paper; 10/80
	14. SPONSORING AGENCY CODE EPA/600/13

15. SUPPLEMENTARY NOTES In proceedings of 5th International Symposium on Polynuclear Aromatic Hydrocarbons, 10/80, Columbus, OH. Paper cleared for publication 3/12/81. pp. 119-131

16. ABSTRACT The paper describes the development and status of a previously described spot test utilizing sensitized fluorescence for the detection and semi-quantitative estimation of polycyclic aromatic hydrocarbons. It also gives data from further laboratory investigation. A series of aromatic hydrocarbon heterocyclic compounds and related substituted compounds were tested to further define test limitations. Data from application of the test to a series of industrial and energy sources are also presented. The test is rapid, very inexpensive, quite sensitive, and reliable.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution	Pollution Control	13B
Spot Tests	Stationary Sources	14B
Aromatic Polycyclic Hydrocarbons		07C
Fluorescence		20F
Measurement		

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	15
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	$9.95

EPA Form 2220-1 (9-73)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

In: Proceedings of the 5th International Symposium on Polynuclear Aromatic Hydrocarbons, 10/80, pp. 119-131, 1981

EPA/600/D-82/082
1982

A SPOT TEST FOR POLYCYCLIC AROMATIC HYDROCARBONS

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Validation Studies

INTRODUCTION

Organic analysis needs for most of today's environmentally related projects may be placed into one of three major categories: methods which are compound specific but not useful for more than one or two classes of compounds, methods which are comprehensive for many classes but not compound specific, and methods which are specific for a single class of compounds and comprehensive for all or most members of that class. Much effort in recent years has been devoted to approaches which are very specific, and some effort has been made to develop comprehensive approaches (1). Both of these approaches have been applied to environmental assessment projects or other broad brush sampling and anaysis programs. Unfortunately, when either of these two types of schemes is correctly and fully applied the cost tends to be quite expensive. Therefore, the need exists for rapid inexpensive tests to screen large numbers of samples for certain compound classes such as polycyclic organic material (POM), polychlorinated biphenyls (PCB), and dioxins. Such tests would be useful to prove or disprove the presence of a certain group of toxic chemicals in a given sample and to thereby possibly obviate the need to perform more specific and costly analysis procedures.

Development and application of a rapid, sensitive, and inexpensive test based on sensitized fluorescence for polycyclic aromatic hydrocarbons (PAH) was described by Smith and Levins at this symposium last year (2). The basic work on the test was adequate and sound (3) and the test has continued to function as a valuable tool. We report here additional experience with the sensitized fluorescence test which further defines its limits and allows further comparison of its performance to other more specific methods.

BACKGROUND

The phenomenon of PAH fluorescence is well documented (4,5). The process of sensitized fluorescence can be thought of as an

energy transfer or energy "pumping" between two materials which amplifies the fluorescence of a component at lower energy levels at the expense of components at higher energy levels. This process is shown by simplified energy-level diagrams in Figure 1. When energy can be transferred from a major component which has no visible spectrum, the sensitized fluorescence can be used in a visual test. Detection limits using this approach have been reported on the order of 1 to 10 pg/spot (2).

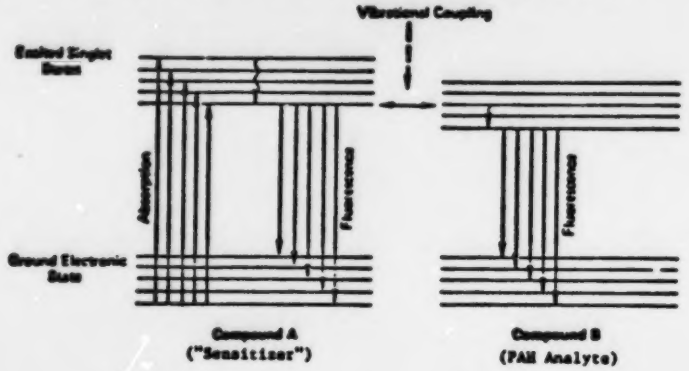

FIGURE 1 SENSITIZED FLUORESCENCE by SINGLET – SINGLET ENERGY TRANSFER

The current effort in EPA's IERL-RTP laboratory centers around evaluation and validation of the spot test in three ways:

1. Does the test respond to most heterocyclic fused organic materials using naphthalene as the sensitizer, or is it limited primarily to polycyclic aromatic hydrocarbons (PAH)? Does the test show a selectivity among PAH material it detects?

2. What compounds or compound classes interfere with sensitized fluorescence? Does quenching or interference occur through adsorption of incident radiation, through interference with the energy transfer between sensitizer and analyte, or through deactivation of the excited state? Can the interference be overcome by dilution of the entire sample?

3. Are there any difficulties in applying the test to real samples of complex mixtures being emitted into the environment?

EXPERIMENTAL

Drummon Microcaps (1 uL), Whatman No. 42 filter paper, and a Model CV 70 ultraviolet cabinet (all acquired from Fisher Scientific) were used in this work.

Aldrich Gold Label naphthalene, prepared at 50 ug/uL in Burdick and Jackson dichloromethne, was used as the sensitizer solution. Experiments in later stages used the same sensitizer at 30 ug/uL with no loss in sensitivity. Analytes for the test evaluation were acquired from Chem Service Inc., West Chester, PA.

The procedure for applying spots and recording fluorescence sensitization has been documented elsewhere (1-3).

Liquid chromatographic separations were performed according to EPA Environmental Assessment Level 1 procedures (4).

Spot Test Procedure

Full details and examples of the use of the sensitized fluorescence spot test are available elsewhere (7). In its simplest form the test is accomplished by marking three circles in a line on a high grade ashless filter paper (e.g., Whatman 42). Each circle is approximately 1 cm in diameter. With a disposable 1 uL pipette, the sample is applied to the unused outside circle and to the center circle. The solvent carrying the sensitizer and the analyte is allowed to evaporate from the paper for approximately 15 seconds. The test spots are then exposed to 254 nm UV radiation, usually in a UV enclosure equipped with viewing port. Differences in intensity between the spots with and without sensitizer are recorded (Figure 2). Appropriate dilutions of the original sample are made on the basis of the first test to determine the approximate concentration of the unknown PAH mixture (2).

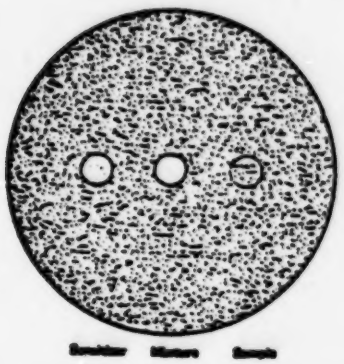

FIGURE 2. Representation of 254 nm Illuminated Spot-Test Series.

Naphthalene was used as the sensitizer in all experiments reported here. The purity of the sensitizer is critical to the effectiveness and detection limit of the test. Off-the-shelf or common ACS grade naphthalene is often contaminated with fluorescent materials: the background from such impurities may be greater than the fluorescence of trace quantities of PAH in unknown analyte mixtures. An effective naphthalene sensitizer (the one used for most of the studies here) is Aldrich Gold Label. It had the lowest level of fluorescence of all the sensitizers tested. The background level of the sensitizer may also be reduced by dilution of the sensitizer solution. In some cases an order of magnitude greater sensitivity was noted with lower concentration sensitizer solution. Use of lower sensitizer concentrations requires rapid observation and recording of response since the fluorescence enhancement disappears as the naphthalene evaporates from the filter paper.

RESULTS

General Response of the Spot Test

Experience with the detection of pure PAH materials using sensitized fluorescence is described by Smith and Levins (2,3).

They report a detection limit of 10 pg per spot for common PAH material such as anthracene or pyrene. A lower detection limit of 1 pg per spot was reported for selected PAH material such as benzo[a]pyrene. Results for compounds like pyrene are similar to those reported by Smith (3). However, results for other compounds, such as anthracene and fluoranthene, show markedly different responses when our result are compared to Levins (2) as shown in Table 1. We attribute these differences

TABLE 1

PAH DETECTION LIMITS IN THE SPOT TEST

	EPA pg/Spot	Others[a] pg/Spot
Dimethylnaphthalene	10^4	--
Acenaphthene	$>10^4$	--
Fluorene	10^4	--
Phenanthrene	10^5	10^2
Anthracene	10^2	10^1
9,12-Dimethylanthracene	10^1	--
Pyrene	10^4	10^4
Fluoranthene	1	10^1
7,12-Dimethylbenzanthracene	10^1	--
3-Methylcholanthrene	10^1	--
Benzo[a]pyrene	--	10^2

a Results from previous work (References 2 and 3).

primarily to differences in purity of the stock PAH supplies and to some extent to differences in intensity of the UV sources. The PAH stock materials are most often contaminated with other fluorescence sensitized materials which can significantly affect spot test results. Our experience shows that PAH material below a molecular weight of 200 has a much higher detection limit than that with molecular weight greater than 200. Naphthalene appears to co-crystalize with PAH material in a manner which favors fluorescence sensitization. One notable exception to this generalization is chrysene whose fluorescence is not sensitized with the addition of naphthalene. The addition of a heteroatom to a fused ring system alters the

spot test response. Compounds such as carbazole and dibenzfuran are not sensitized by the addition of naphthalene. Other higher molecular weight heterocyclic fused aromatic compounds have also been evaluated. When the heterocyclic ring divides the fused hydrocarbon rings into units of two or less, the compounds fluoresce but are not sensitized, e.g., benzoquinoline. Indirect evidence from environmental samples suggest that heterocyclic fused aromatic compounds with three or more fused aromatic hydrocarbon rings joined to the heterocyclic ring will respond in the sensitized fluorescence spot test.

In addition to pure PAH compounds, other substances from a variety of chemical classes were used to evaluate the test. A partial list of these compounds and their empirical formulas are given in Table 2. None of these compounds was positive in the spot test.

TABLE 2

SELECTED MATERIAL NEGATIVE IN THE PAH SPOT TEST

Carbazole	$C_{12}H_9N$
Isoquinoline	C_7H_7N
Hydroxyquinoline	C_9H_7NO
Azobenzene	$C_{12}H_{10}N_2$
pp'-DDE	$C_{14}H_8Cl_4$
Biphenyl	$C_{12}H_{10}$
Bibenzyl	$C_{14}H_{14}$
p-terphenyl	$C_{18}H_{14}$

Interfering Compounds

Potential interference, for the purpose of this study, occurs when application of an analyte extinguishes fluorescence and causes a dark spot to occur in place of the white filter paper background. Interference is caused by either or both of two mechanisms. It may occur if the interfering compound is in high concentration and has sufficient extinction to absorb

the incident UV radiation. It may also occur if a compound interferes with the energy transfer from the sensitizer to the analyte. Dilution of the sample with inert solvent should circumvent the first mechanism, but usually not the second. Examples of both mechanisms have been encountered.

Table 3 shows a partial list of compounds found to interfere with low level sensitized fluorescence. Of those listed, phthalates and PCBs are of interest to EPA because of their common occurrence in environmental samples. Therefore,

TABLE 3

POTENTIAL INTERFERENCES TO THE SENSITIZED FLUORESCENCE TEST

Hydroxyquinoline	C_9H_7ON
Benzothiazole	C_7H_5NS
2 Imidazolidinethione	$C_3H_6N_2S$
2 Methylbenzothiazol	C_8H_7NS
Aroclor 1254	—
Trinitrotoluene[a]	$C_7H_5O_6N_3$
Trinitrotoluene[b]	$C_7H_5O_6N_3$
Dibutyl Phthalate	$C_{16}H_{22}O_4$
Dioctyl Phthalate	$C_{22}H_{38}O_4$
Methanol Extract of TYDEX [a]	—

[a] From Smith (3)

analyses were performed to determine if interferences from these two classes of compounds can be circumvented by dilution of the entire sample or if they present a major difficulty when using the sensitized fluorescence test. Results of these analyses show that phthalates, which appear to interfere via absorption of incident radiation, are easily overcome with dilution. PCB material such as Aroclor 1254 interfere by a mechanism which cannot be overcome by dilution. Fortunately PCBs interfere only at high concentrations. In experiments using Aroclor 1254 and fluoranthene, spots containing only PCBs appeared black. Addition of sensitizer and fluorescent material caused no change in appearance. However the ratio of PCBs to fluoranthene which totally inhibits sensitized

fluorescence is approximately 50,000 to 1. In such samples the PCBs will probably be the prime environmental concern.

The conclusion from this limited investigation and from the results reported by Smith (3) is that selected materials in high concentrations may produce false negative results. Dilution does not aid detection of sensitized fluorescence when the interference involves inhibition of energy transfer. Because the sensitized fluorescence test has such low detection limits, only extremely high interferent concentration will cause difficulties for the test. Some additional effort remains to be brought to bear on the interference noted by Smith (1) in order to determine if the ratio of nitrated organic material to PAH which inhibits sensitized fluorescence is sufficiently small to cause difficulties from combustion related environmental samples.

Environmental Samples

To further evaluate the spot test two environmental samples containing complex mixtures of organic material were screened using the spot test. Conventional chemical analyses including liquid chromatographic and gas chromatographic separation followed by low resolution mass spectrometry were applied to the samples according to EPA Level 1 Procedures (4).

The first sample described here was collected from the off gas of a roofing pitch vat heated to 156°C. Samples were collected through a stainless steel hood positioned over one of the vat openings. Ambient air was drawn across the hot pitch and the condensable fraction was collected from the walls of a stainless steel condensate pot. The resulting sample was totally soluble in methylene chloride.

The spot for this sample test was positive with strong sensitized fluorescence on the material diluted to 1.5 ug/uL. Below this concentration no sensitized fluorescence could be detected. This corresponds to a PAH concentration of 0.6 to 6% in the original sample. Level 1 organic analysis as shown in Figure 3 includes the use of liquid chromatography, infrared spectroscopy, and low resolution spectrometry, was performed on this sample. A summary of the results is shown in Table 4. Mass spectrometry analysis of the seven resulting fractions indicated that 60% of the sample was PAH, ranging in molecular weight from 178 to 278.

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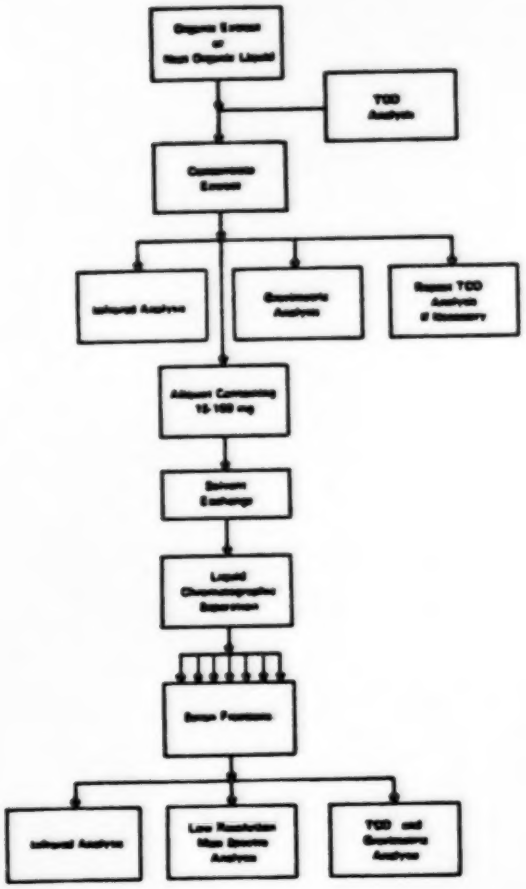

FIGURE 3. ORGANIC ANALYSIS METHODOLOGY.

TABLE 4

ORGANIC EXTRACT SUMMARY TABLE OF ROOFING PITCH VOLATILES

	9	639	475	115	7	87	8	1160
	0	125	57	60	2	70	0	270
	7	235	324	38	0	30	0	600

	100/0							0
	10/1	100/400	100/430	10/9.6				840
		10/40	10/43					83
				100/95		100/87		100
				10/9.6				9.6
		10/40						40

Based on the range of sensitivities shown by PAH compounds in the spot test (1 - 10^4 pg/spot), agreement for this sample between the conventional methods and sensitized fluorescence methods is within an order of magnitude.

A second example of application of the spot test involves testing the complex mixture and several LC fractions from a sample of coke main condensate. The sample was collected from the main condensate duct leading from a coking oven. This tar-like liquid is usually accumulated and burned in another area of the process. This sample contained a small percentage of particulate material which is probably best defined as coal dust. The remainder of the sample was 100% soluble in dichloromethane.

The spot test was positive on the whole sample diluted to a concentration of 11 pg/uL. This result indicated that from 10 to 100% of the sample is PAH material. Level 1 analysis indicated that 65% of the sample was PAH and a major portion of the remaining 35% was heterocyclic nitrogen compounds (Table 5).

TABLE 5

ORGANIC EXTRACT SUMMARY TABLE FOR COKE OVEN VOLATILES

[illegible] COKE OVEN VOLATILES

	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	0	357	357	95	10	155	5	860
[illegible]	0	83	11	5	0	0	0	110
[illegible]	0	74	260	80	14	110	4	530

[illegible] [illegible] - mg/kg

[illegible]		[illegible]	[illegible]	[illegible]	[illegible]			[illegible]
[illegible] Organic Hydrocarbons		100/100	100/357	10/9	10/1.5			500
[illegible] S Compounds		10/19						29
[illegible] N Compounds				100/83	100/15	100/70		170
Olefins				10/8	10/1.5			9
Phenols						10/7		7
Esters						100/70	100/4	74
Esters							10/0.4	0.4
Carboxylic Acids							10/0.4	0.4
Unclassified						10/7	10/0.4	0.4

To determine if the heterocyclic compounds were also positive in the PAH fluorescence sensitization test, each of the seven Level 1 fractions was diluted to a known concentration. Since fraction 1, which would normally contain paraffins, did not contain any measurable mass (Table 5); the contents of this fraction were diluted to 1 mL volume for testing. Spot test results for the seven fractions are shown in Table 6. As anticipated, fractions containing PAHs were positive in the test. However, fractions not generally expected to contain PAHs were also positive. For instance, fraction 1, with no weighable organic, was positive with sensitization in the fluorescence test indicating 1 to 10 ng of PAH possible in this fraction. This is reasonable based on a detection limit of 20 ng for the confirmatory techniques used.

TABLE 6

SENSITIZED FLUORESCENCE RESULTS COKE MAIN FRACTIONS

Fraction	Level 1 Results % PAH	Fluorescence Spot Test Results, % PAH
1	11	+ <1
2	90	+0.01
3	91	10-100
4	8	10-100
5	--	10-100
6	0	10-100
7	--	2-17

Fraction 7 is particularly interesting. The sensitized fluorescence test indicates 6.5 ng/uL of PAH which is less than 1% of the total organic material found in this fraction. Since this amount is well below the detection limit of the mass spectrometer, it is not surprising to find that PAH material was not detected in the Level 1 results for this fraction.

SUMMARY

The results from all sources, including the present work using the sensitized fluorescence spot test, support the use of this test to detect PAH material. It has been shown to have wide application to environmental samples from various media. It also has been shown to have a detection limit for PAH which is usually better than conventional spectrometric techniques. The quantitative agreement between conventional methods and the sensitized fluorescence test is also shown. The sensitized spot test is an inexpensive approach (~$10/sample) for screening a wide variety of samples for PAH material. It is recommended for detecting the presence of PAHs and for use as an indicator of the need for subsequent quantitative analysis of individual PAH compounds.

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